On the Etiology of Keratocyte Loss during Contact Lens Wear

Panagiotis Kallinikos and Nathan Efron

Purpose. To employ confocal microscopy to investigate the etiology of keratocyte loss after short-term contact lens wear by monitoring quantitative changes in keratocyte density.

Methods. Twenty neophyte subjects aged 26 ± 3 years participated in the study, which was conducted over the course of three experimental sessions. In the first session, one eye of each subject was fitted with a silicone hydrogel contact lens, and the other eye served as the control. Both corneas were exposed to an anoxic environment for 2 hours. Ultrasound pachometry and confocal microscopy were performed on both eyes at baseline, immediately after the experiment and 2 hours post experiment. This procedure was repeated after 72 hours, but in this case one eye of each subject was fitted with a hyper-Dk rigid contact lens, and the fellow eye served again as the control. In the third experimental session, each subject was asked to periodically rub one eye only. Tear samples collected from the rubbed and control eyes were assayed for epidermal growth factor (EGF), hepatocyte growth factor (HGF), and interleukin (IL)-8.

Results. The increase in corneal thickness was similar in the experimental and control eyes. Both anterior and posterior keratocyte densities decreased in the experimental eyes compared with the control eyes, in all sessions. EGF and IL-8 concentrations were increased in the rubbed eyes compared with the control eyes.

Conclusions. It is hypothesized that the mechanical stimulation of the corneal surface, due to the physical presence of a contact lens, induces the release of inflammatory mediators that cause keratocyte dysgenesis or apoptosis. (*Invest Ophthalmol Vis Sci.* 2004;45:3011–3020) DOI:10.1167/iovs.04-0129

The corneal stroma is a dynamic structure consisting mainly of keratocytes within an extracellular matrix of collagen fibers and proteoglycans. Keratocytes play an active role in maintaining corneal transparency, health, and structural stromal stability, by regulating collagen fibril size and spacing within the proteoglycan matrix. They are connected by gap junctions forming a communicating network within the corneal stroma.[1] Keratocyte morphology and ultrastructural details have been studied extensively, using histologic methods,[2,3] electron microscopy,[4] and fluorescent markers used with light microscopy.[5] Keratocyte density and distribution have been examined by using biochemical measurements of the DNA content within the corneal stroma.[6–8]

Bergmanson and Chu[9] used electron microscopy to demonstrate that posterior stromal keratocytes degenerate and die after acute polymethylmethacrylate (PMMA) contact lens wear in primates. This work created an impetus for further work exploring the effects of contact lens wear on keratocyte populations. The recent availability of the clinical confocal microscope[10] allows the in vivo human keratocyte network to be examined both qualitatively and quantitatively. Bansal et al. (*IOVS* 1997;38:ARVO Abstract S674), Jalbert and Stapleton[11] and Efron et al.[12] showed that keratocyte density in the human cornea decreases during contact lens wear. The contact-lens–induced keratocyte loss observed by these investigators was attributed to three possible etiologies: hypoxia, cytokine-mediated effects, and mechanically induced effects. However, in later work, Patel et al.[13] questioned the findings of the previous studies. They demonstrated that long-term daily contact lens wear has no effect on keratocyte density. Efron et al.[14] emphasized the importance of considering the levels of corneal residual edema at the time of undertaking confocal microscopy, when measuring keratocyte density, since the unidimensional volumetric stromal expansion and the degradation of image quality may have a confounding effect on the interpretation of the data.

To explore the etiology of keratocyte loss during contact lens wear, we used ultrasound pachometry and confocal microscopy to monitor changes in keratocyte density after short-term contact lens wear. We also sought to determine the mechanism that could be responsible for any alterations observed by obviating the effects of corneal edema.

Methods

Patient Selection

Twenty human subjects participated in the study. They comprised 7 men and 13 women, aged 26 ± 3 (mean ± SD) years. None of the subjects had worn contact lenses previously or had had refractive surgery. The exclusion criteria were the presence of ocular and/or systemic disease and allergy to the anesthetic or eye gel used during the confocal microscopic examination. The number of subjects recruited for the study was determined using power analysis. A sample size of 20 subjects would have 81% power to detect an effect size of 0.67 (a clinically significant difference in keratocyte density of 100 with a typical standard deviation for this measure of ±150, using a paired *t*-test with a 5%, two-sided significance level). The research adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all subjects after explanation of the nature and possible consequences of the study. The protocol used was approved by the Local Research Ethics Committee (Central) of Manchester Health Authority.

Experimental Protocol

The study was conducted over the course of three experimental sessions. In the first session, one eye of each subject—randomly determined by the subject—was fitted with a silicone hydrogel (Si-H)

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TABLE 1. Lens Details

	Focus Night & Day	Menicon Z-alpha
Manufacturer	CIBA Vision Corporation	Menicon Co. Ltd.
Material name	Lotrafilcon A	Silicone-containing methacrylate
Material type	Silicone hydrogel	Hyper-Dk rigid
Dk (barrers)	140	163
Diameter (mm)	13.8	9.2 and 9.6
Back Vertex Power (D)	−3.00	−3.00
Back optic zone radius (mm)	8.4 and 8.6	7.2 to 8.4 in 0.05-mm steps

contact lens (Focus Night & Day; CIBA Vision Corp., Duluth, GA), and the fellow eye served as the control. The specifications of the lenses fitted are summarized in Table 1. The basic fitting philosophy was to allow for maximum possible movement consistent with good comfort. Both eyes were exposed to an anoxic environment (100% humidified nitrogen) through modified swimming goggles, for 2 hours. After 2 hours of gas exposure, the goggles and the lens were removed. Ultrasound pachometry and confocal microscopy of the central corneas of both eyes were conducted at baseline (before lens wear and nitrogen exposure), immediately after the end of the anoxia exposure period and cessation of lens wear, and 2 hours after the experiment (post experiment).

In the second experimental session the same protocol was followed as in the first study, but in this case a Menicon Z-alpha (Menicon Co. Ltd., Nagoya, Japan) rigid contact lens was worn in one eye only of each subject, with the other eye serving again as the control. The characteristics of these lenses are also summarized in Table 1.

In the third experimental session, each of the 20 subjects was asked to mechanically stimulate one cornea only, by slightly rubbing the eye on the anterior surface of the closed eyelid with the finger, performing smooth circular movements. The subjects were asked to rub the same eye only for 10 seconds, every 1 minute, for a total period of 30 minutes (i.e., 30, 10-second eye rubs). Both eyes remained open except when eye rubbing was taking place. Bilateral basal tear fluid samples were collected from each subject after the end of the 30-minute period, as described later. Ultrasound pachometry and corneal confocal microscopy were performed again at baseline, immediately after the 30-minute period of eye rubbing, and 2 hours post experiment.

Each experimental session was performed at least 4 hours after awakening, to avoid the confounding effects of immediate postsleep corneal edema. The time interval between two consecutive experimental sessions for the same subject was no less than 72 hours.

Ultrasound Pachometry

Central corneal thickness measurements were obtained using an ultrasonic pachometer (model 850; Allergan-Humphrey, San Leandro, CA), as previously described.[14] The probe was carefully positioned perpendicular to the visual axis, and six readings were obtained in each eye. The mean ± SD of the corneal thickness of each eye was calculated. The percentage change in corneal thickness immediately after the exposure period and 2 hours post cessation was calculated.

Confocal Microscopy

Patients underwent examination with a corneal in vivo confocal microscope (model P4, Confoscan; Tomey, Erlangen, Germany), in accordance with our established protocol.[14–16] Patients were instructed to gaze straight ahead, and a fixation target was attached to the chin and head rest to facilitate steady fixation. Several scans of the entire depth of the central cornea were made, to acquire images of all corneal layers with a lateral resolution of approximately 1 to 2 μm and final image size of 768 × 576 pixels. The three best-quality images of the anterior and posterior stroma were chosen for investigation after each examination. Images of the anterior stroma were considered to be those image frames captured immediately posterior to the anterior limiting membrane (Bowman's membrane), and images of the posterior stroma were taken to be those immediately anterior to the posterior limiting membrane (Descemet's membrane).

Data Analysis: Image Processing

The analysis of the data was performed in a masked and randomized manner. Three hundred sixty examinations were performed in total, each of which was assigned a random number between 1 and 360. The quantitative analysis of the images was performed after the completion of all the examinations, and the examiner was masked to the condition of the eye (lens–wearing/mechanically stimulated or control) and the experimental session (effects of silicone hydrogel lens, rigid lens, and eye rubbing) to which each image corresponded.

Digital images of the corneal stroma were analyzed using the confocal microscope's integrated software (Confo-commander v 2.7.1; Tomey). Keratocyte density was determined in a central region of interest of a fixed arbitrary size (159 × 131 μm) drawn over the image, wherein the cells were counted manually and reported as cells per square millimeter. The mean anterior keratocyte density (AKD) of the three analyzed anterior stromal frames and the mean posterior keratocyte density (PKD) of the three analyzed posterior stromal frames were measured in the Si-H-lens–wearing and control eyes, in the rigid-lens–wearing and control eyes, and in the rubbed and control eyes. The percentage change in AKD and PKD, both immediately after the exposure period and 2 hours post experiment, was calculated. The data obtained represented the total observed change in keratocyte density, including the effects of corneal edema and the effects of any additional etiological factor. The total observed change in keratocyte density was corrected for the effects of corneal swelling, according to the binomial expansion model developed by Efron et al.[14] The binomial expansion model predicted that, with low levels of corneal edema (<15%), the magnitude of the apparent loss of keratocytes should be roughly equivalent to the level of edema. The percentage increase in corneal thickness was subtracted from the total observed change in keratocyte density. The values obtained represented the "real" change in keratocyte density, which could not be explained by the artifact caused by the volumetric tissue expansion due to the presence of corneal swelling. The statistical analysis of the data performed between the experimental and control eyes in the three experimental sessions is described later.

The contrast of anterior and posterior confocal images was also evaluated, using image-processing software (Scion Corporation, Frederick, MD). An approximation of the contrast of each image was obtained by subtracting the background intensity from the mean intensity of the image and dividing by the background intensity. The result was multiplied by 100%. The mean and background intensities were estimated with a readily available macro called "integrated density," after inverting the gray values of the pixels in the image. The background intensity is represented by the modal gray value and the mean intensity is represented by the average gray value. Specifically, the mean contrast of the three anterior and the three posterior stromal images obtained from each examination was calculated. The percent-

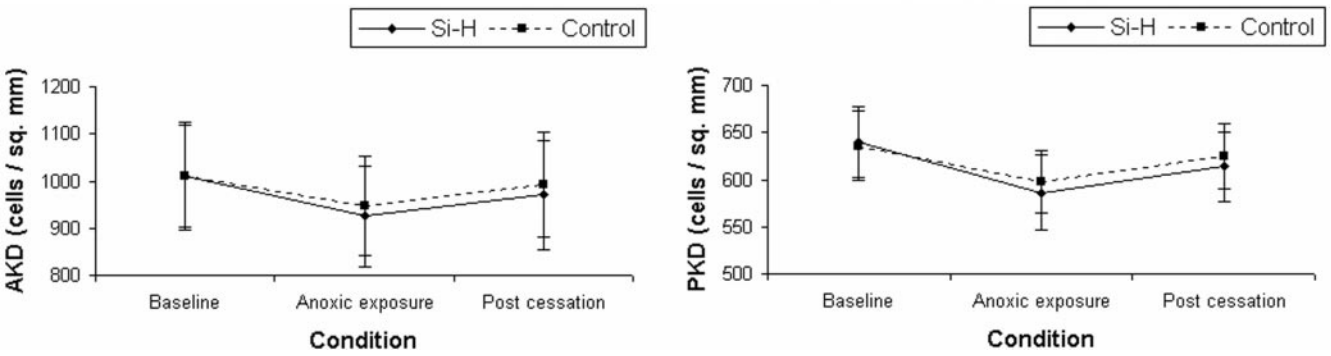

FIGURE 1. Anterior (*left*) and posterior (*right*) stromal keratocyte density (mean ± SD) in the Si-H-lens–wearing and control eyes at baseline, immediately after the exposure period, and 2 hours post experiment; $n = 20$ for each data point.

age change in the contrast of confocal images immediately after the experiment and 2 hours post experiment was calculated.

Tear Fluid Collection

Tear samples were collected with a 50-μL fire-polished, graded microcapillary tube, as previously described,[17] from the inferior temporal tear meniscus of each participant, without corneal anesthesia, taking care to ensure that no contact was made with the lid margin and corneal surface. The samples were immediately transferred to Eppendorf tubes (Eppendorf AG, Hamburg, Germany) and stored at −70°C until assayed for epidermal growth factor (EGF), hepatocyte growth factor (HGF), and interleukin (IL)-8. The tear fluid flow in the collection capillary was calculated by dividing the volume of the tear fluid sample by the tear fluid collection time (in microliters per minute). The tear-flow–corrected concentration (i.e., rate of release) was calculated by multiplying the concentration in the sample (in picograms per milliliter) by the tear fluid flow in the collection capillary (in microliters per minute), as previously described,[18–21] to compensate for the dilution effect caused by a possible hypersecretion of tears after the mechanical stimulation of the cornea.

The capillary method of collecting tears contained a possible source of error. Despite the gentle aspiration technique with fire polished capillaries, some of the subjects experienced a slight foreign-body sensation during tear collection. In these subjects, reflex tearing may have been triggered and mixture with the basal secretion may have occurred. However, despite the inaccuracy of the capillary tube method, we consider it a suitable technique for collecting tears for quantifying the concentration of mediators in tear fluid.[20]

Cytokine Assays

Tear samples were assayed for EGF, HGF, and IL-8 with commercially available ELISA kits (R&D Systems Europe Ltd., Abingdon, UK). The samples were centrifuged at 13,000 rpm for 2 minutes. The supernatant was transferred in new Eppendorf tubes. Sample preparation and analysis followed the protocols supplied by the manufacturers. Because tear samples were available in small volumes, each sample was diluted to 1:25. The minimum detectable unit of ELISA was 0.7 pg/mL for EGF, 40 pg/mL for HGF, and 10 pg/mL for IL-8. The concentration of EGF, HGF, and IL-8 in each sample (pg/mL) was determined, according to the corresponding absorbance of each sample.

Statistical Analysis

Data were analyzed on computer (SPSS, ver. 10.0, for Windows; SPSS Science, Chicago, IL). A one-sample *t*-test was conducted to investigate whether the mean changes in corneal thickness, AKD, and PKD and contrast of anterior and posterior confocal images were significantly different from zero, for each eye category, at each experimental session. A two-factor, within-subject, repeated-measures analysis of variance was conducted to compare the percentage change in corneal thickness, AKD, and PKD and contrast of anterior and posterior confocal images, between the experimental (lens-wearing or rubbed) and control eyes in all three experimental sessions. The "experimental session" was the first within-subject factor with three levels (effects of Si-H lenses, effects of rigid lenses, effects of eye rubbing). The "eye category" was the second within-subject factor, with four levels (experimental eyes after the exposure period and control eyes after the exposure period, and experimental eyes 2 hours post cessation and control eyes 2 hours post cessation). $P \leq 0.05$ was taken as the threshold of statistical significance. Bonferroni adjustment of the confidence interval was used when appropriate. Further to the assessment of these factors, the interaction between the experimental session and the eye category was analyzed.

A multivariate analysis of variance (m-ANOVA) was conducted to compare the concentration and rate of release of EGF, HGF, and IL-8 between the tear fluid samples obtained from the mechanically stimulated eyes and those obtained from the control eyes. When the normality assumption was not satisfied, a Kruskal-Wallis test was conducted. Significant differences between the two groups were established at $P \leq 0.05$.

RESULTS

Corneal Thickness

The absolute AKD and PKD in the Si-H-lens–wearing and control eyes, in the rigid-lens–wearing and control eyes, and the rubbed and control eyes are presented in Figures 1, 2 and 3, respectively. The key statistics for all the parameters assessed are presented in Table 2, and the pair-wise comparisons between the experimental and control eyes, both immediately and 2 hours after the experiment, are summarized in Table 3. The percentage change in corneal thickness in all eye groups in all three experimental sessions is presented in Figure 4. In the first experimental session, the increase in corneal thickness was significantly different from zero in the Si-H-lens–wearing eyes and control eyes, both immediately after ($t = 17.9$, $P < 0.001$ and $t = 22.1$, $P < 0.001$, respectively) and 2 hours after ($t = 4.1$, $P = 0.001$ and $t = 3.7$, $P = 0.001$, respectively) the anoxia exposure period. In the second experimental session, the increase in corneal thickness was significantly different from zero in the rigid-lens–wearing and control eyes, both immediately after ($t = 20.6$, $P < 0.001$ and $t = 5.9$, $P < 0.001$, respectively) and 2 hours after ($t = 20.5$, $P < 0.001$ and $t = 6.1$, $P < 0.001$, respectively) the anoxia exposure period. In the third experimental session, the increase in corneal thickness was different from zero in the rubbed eyes and control eyes, only immediately after the experiment ($t = 8.6$, $P <$

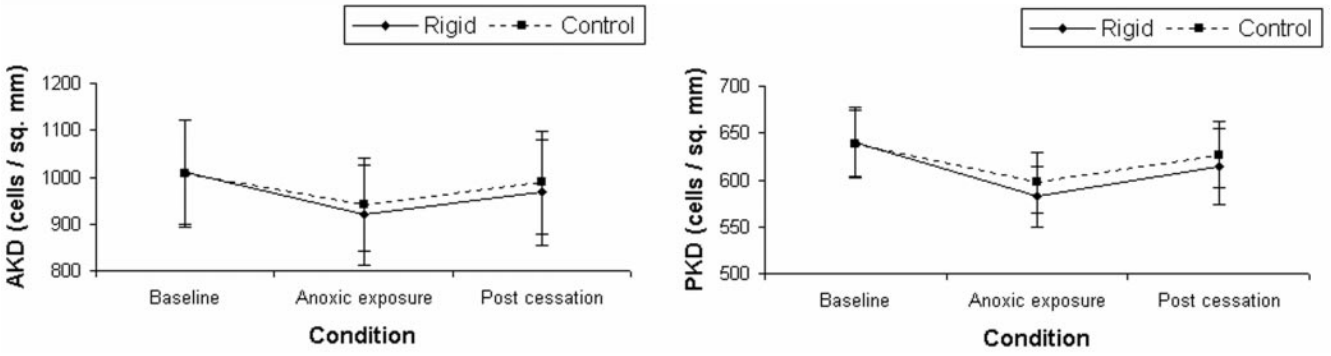

FIGURE 2. Anterior (*left*) and posterior (*right*) stromal keratocyte density (mean ± SD) in the rigid-lens–wearing and control eyes at baseline, immediately after the exposure period and 2 hours post experiment; $n = 20$ for each data point.

0.001 and $t = 7.9$, $P < 0.001$, respectively). The increase in corneal thickness was not significantly different from zero in the rubbed and control eyes 2 hours post experiment ($t = 1.5$, $P = 0.159$ and $t = 2.1$, $P = 0.056$, respectively). Statistically significant differences were established between the three experimental sessions for the increase in corneal thickness ($F = 103$, $P < 0.001$). The overall increase in corneal thickness was greater in the first experimental session than in the third session ($P < 0.001$) and in the second session than in the third session ($P < 0.001$). There were no statistically significant differences between the first and the second experimental sessions ($P = 0.999$). There were statistically significant differences observed between the four eye categories for the increase in corneal thickness ($F = 234.7$, $P < 0.001$). Specifically, there were statistically significant differences in the increase in corneal thickness immediately after the exposure period and 2 hours post cessation in the experimental ($P < 0.001$) and control eyes ($P < 0.001$). However, there were no statistically significant differences between the lens-wearing/rubbed and control eyes both immediately after the exposure period ($P = 0.832$) and 2 hours post cessation ($P = 0.999$). A significant interaction between the experimental sessions and the eye categories was also observed ($F = 60.57$, $P < 0.001$). Post hoc analysis confirmed that there were no statistically significant differences in the increase in corneal thickness between the Si-H-lens–wearing and control eyes, both immediately after the exposure period ($P = 0.999$) and 2 hours post cessation ($P = 0.999$), between the rigid-lens–wearing and control eyes both immediately after the exposure period ($P = 0.999$) and 2 hours post cessation ($P = 0.999$), and between the rubbed and control eyes, both immediately after the 30-minute period ($P = 0.999$) and 2 hours post experiment ($P = 0.999$).

Anterior Keratocyte Density

The percentage change in corrected AKD in all eye groups in all three experimental sessions is presented in Figure 5. In the first experimental session, the reduction in AKD was significantly different from zero in the Si-H-lens–wearing eyes and control eyes, immediately after the anoxic exposure period ($t = -9.2$, $P < 0.001$ and $t = -2.2$, $P = 0.043$, respectively). Two hours post experiment, the reduction in AKD was significantly different from zero, only in the Si-H-lens–wearing eyes, but not in the control eyes ($t = -6.7$, $P < 0.001$ and $t = -0.7$, $P = 0.493$, respectively). In the second experimental session, the reduction in AKD was significantly different from zero in the rigid-lens–wearing eyes, both immediately after the experiment and 2 hours post experiment ($t = -9.1$, $P < 0.001$ and $t = -5.9$, $P < 0.001$, respectively), whereas it was not significantly different from zero in the control eyes, either immediately after the experiment or 2 hours post experiment ($t = -2.1$, $P = 0.056$ and $t = -0.7$, $P = 0.504$, respectively). In the third experimental session, the reduction in AKD was significantly different from zero in the rubbed eyes and control eyes, immediately after the anoxic exposure period ($t = -10.8$, $P < 0.001$ and $t = -2.4$, $P = 0.026$, respectively). Two hours post experiment, the reduction in AKD was significantly different from zero, only in the Si-H-lens–wearing eyes, but not in the control eyes ($t = -8.9$, $P < 0.001$ and $t = -1.0$, $P = 0.328$, respectively). The overall change in AKD was similar in all three experimental sessions ($F = 0.04$, $P = 0.958$), showing that the effects of Si-H lens wear, rigid lens wear, and eye rubbing on AKD are of a similar magnitude. However, there were significant differences between the four eye groups ($F = 34.93$, $P < 0.001$), with no significant interaction between the

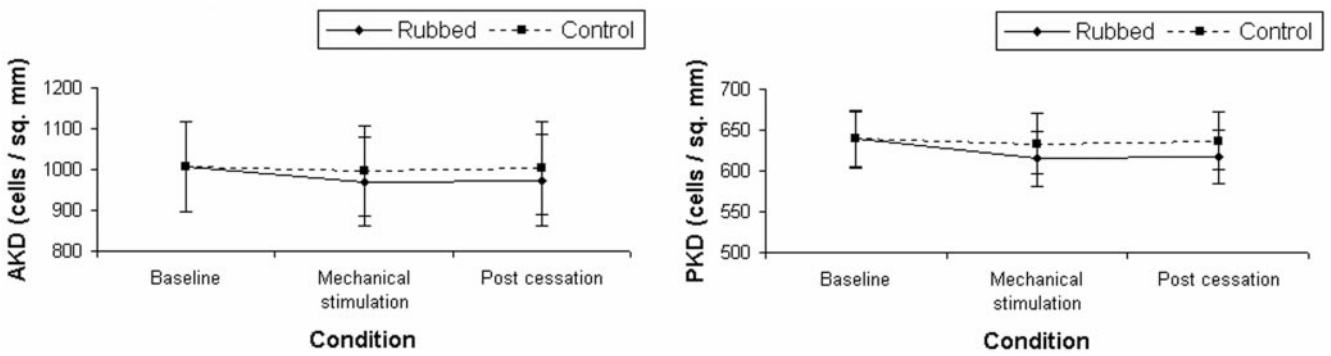

FIGURE 3. Anterior (*left*) and posterior (*right*) stromal keratocyte density (mean ± SD) in the rubbed and control eyes at baseline, immediately after the exposure period and 2 hours post experiment; $n = 20$ for each data point.

TABLE 2. Key Statistics for Corneal Thickness, Anterior and Posterior Keratocyte Densities, and Contrast of Anterior and Posterior Stromal Images

Parameters	Source	F-value	Significance
Corneal Thickness	Experimental session	103.00	$P < 0.001$
	Eye category	234.70	$P < 0.001$
	Interaction	60.57	$P < 0.001$
Anterior keratocyte density	Experimental session	0.04	$P = 0.958$
	Eye category	34.93	$P < 0.001$
	Interaction	0.79	$P = 0.560$
Posterior keratocyte density	Experimental session	0.12	$P = 0.890$
	Eye category	36.71	$P < 0.001$
	Interaction	2.02	$P = 0.790$
Contrast of anterior stromal images	Experimental session	0.92	$P = 0.408$
	Eye category	0.36	$P = 0.784$
	Interaction	0.05	$P = 0.999$
Contrast of posterior stromal images	Experimental session	0.79	$P = 0.458$
	Eye category	0.16	$P = 0.925$
	Interaction	0.02	$P = 0.999$

Significant differences were established at $P = 0.05$.

experimental sessions and the eye groups ($F = 0.79$, $P = 0.560$). Post hoc analysis confirmed that there were statistically significant differences for the percentage change in AKD between the experimental and control eyes, both immediately after the exposure period ($P < 0.001$) and 2 hours post experiment ($P < 0.001$). There were no statistically significant differences between the change in AKD immediately after the experiment and the change 2 hours post experiment, in the experimental eyes ($P = 0.999$), indicating that there was no significant recovery toward baseline in AKD after 2 hours.

Posterior Keratocyte Density

The percentage change in corrected PKD in all eye groups in the three experimental sessions is presented in Figure 6. In the first experimental session, the reduction in PKD was significantly different from zero, only in the Si-H-lens–wearing eyes, both immediately after the anoxic exposure period and 2 hours post experiment ($t = -7.6$, $P < 0.001$ and $t = -4.9$, $P < 0.001$, respectively), but not in the control eyes, either immediately after the anoxic exposure period or 2 hours post experiment ($t = -1.4$, $P = 0.183$ and $t = -0.7$, $P = 0.474$, respectively). Similarly, in the second experimental session, the reduction in PKD was significantly different from zero, only in the rigid-lens–wearing eyes, both immediately after the anoxic exposure period and 2 hours post experiment ($t = -8.1$, $P < 0.001$ and $t = -5.2$, $P < 0.001$, respectively), but not in the control eyes, either immediately after the anoxic exposure period or 2 hours post experiment ($t = -2.0$, $P = 0.058$ and $t = -0.7$, $P = 0.511$, respectively). In the third experimental session, the reduction in PKD was significantly different from zero, only in the rubbed eyes, both immediately after the anoxic exposure period and 2 hours post experiment ($t = -4.5$, $P < 0.001$ and $t = -8.7$, $P < 0.001$, respectively), but not in the control eyes, either immediately after the anoxic exposure period or 2 hours post experiment ($t = -0.9$, $P = 0.364$ and $t = -0.6$, $P = 0.561$, respectively). The percentage change in PKD was not significantly different in the three experimental sessions ($F = 0.12$, $P = 0.890$), indicating that Si-H lens wear, rigid lens wear, and eye rubbing have similar effects on PKD. Statistically significant differences were established between the eye categories ($F = 36.71$, $P < 0.001$), with no significant interaction between the sessions and the eye groups ($F = 2.02$, $P = 0.790$). Pair-wise comparisons revealed significant differences for the percentage change in PKD between the experimental and control eyes, both immediately after the experiment ($P < 0.001$) and 2 hours post experiment ($P < 0.001$). No statistically significant differences were found between the percentage change in PKD immediately after the exposure period and 2 hours post experiment in the experimental eyes ($P = 0.999$), demonstrating that the reduction in PKD was maintained 2 hours post cessation.

Contrast of Anterior Stromal Images

The percentage change in the contrast of anterior confocal images is presented in Figure 7. The reduction in contrast of anterior stromal images was not significantly different from zero in any of the eye categories at any of the three experimental sessions. There were no statistically significant differences observed between the three experimental sessions for the percentage change in the contrast of anterior stromal images ($F = 0.92$, $P = 0.408$). No significant differences were established between the eye groups ($F = 0.36$, $P = 0.784$), with no interaction between the experimental sessions and the eye categories ($F = 0.05$, $P = 0.999$).

TABLE 3. Pairwise Comparisons between the Experimental and the Control Eyes, both Immediately after and 2 Hours after the Experiment

Parameter	Time Point	*P*
Corneal thickness	Immediately after lens wear period	$P = 0.832$
	2 h post-experiment	$P = 0.999$
Anterior keratocyte density	Immediately after lens wear period	$P < 0.001$
	2 h post-experiment	$P < 0.001$
Posterior keratocyte density	Immediately after lens wear period	$P < 0.001$
	2 h post-experiment	$P < 0.001$

Significant differences were established at $P = 0.05$.

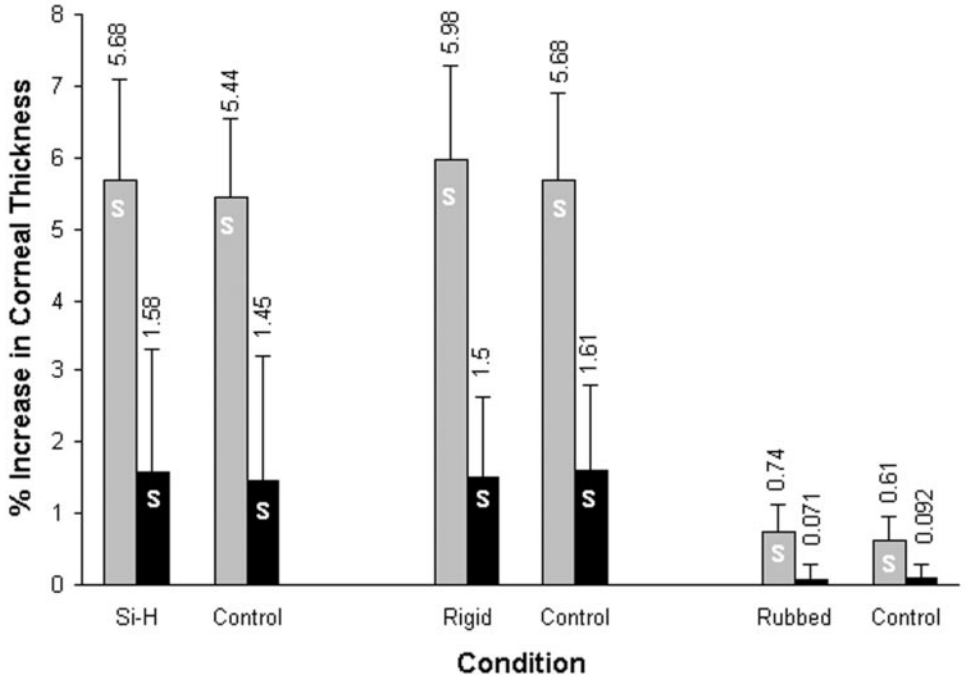

FIGURE 4. The percentage change in corneal thickness relative to baseline for all eye groups in the three experimental sessions. (□) The mean change in corneal thickness immediately after the exposure period; (■) the change in corneal thickness 2 hours post experiment. The height of the histograms corresponds to the mean percentage change in corneal thickness, which is also presented numerically. The error bars represent the corresponding standard deviations. 'S' indicates that the increase in corneal thickness was significantly different from zero ($P < 0.05$). There were no statistically significant differences established for the change in corneal thickness between the experimental and control eyes, both immediately after the experiment and 2 hours post experiment.

Contrast of Posterior Stromal Images

The percentage change in the contrast of posterior stromal images for the three experimental sessions in all eye groups is presented in Figure 8. The reduction in contrast of posterior stromal images was not significantly different from zero in any of the eye categories, at any of the three experimental sessions. The percentage change in the contrast of posterior stromal images was similar in the three experimental sessions ($F = 0.79$, $P = 0.458$). No significant differences were found for the decrease in the contrast of posterior stromal images between the eye groups ($F = 0.16$, $P = 0.925$), with no significant interaction between the sessions and the eye groups ($F = 0.02$, $P = 0.999$).

Concentrations and Rates of Release of EGF, HGF, and IL-8

The descriptive statistics for EGF and IL-8 concentrations and rates of release in all tear fluid samples are given in Table 4. EGF and IL-8 concentrations in the two groups of eyes are presented in Figure 9. HGF was not detected in any of the tear

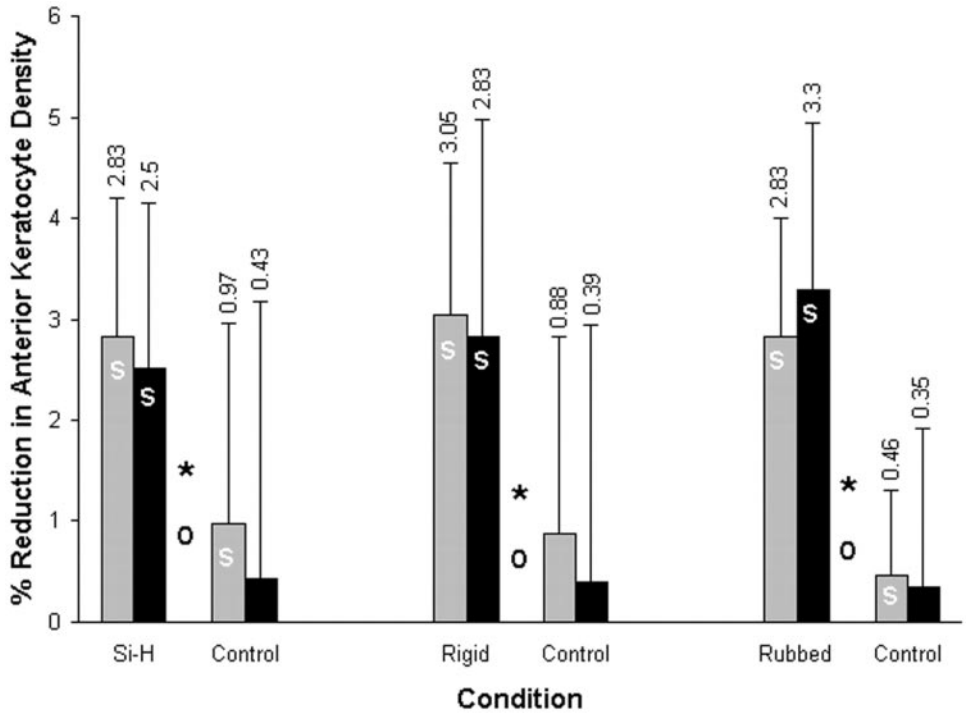

FIGURE 5. The percentage change in AKD relative to baseline in all eye groups in the three experimental sessions. (□) The mean change in AKD immediately after the exposure period and (■) the change in AKD 2 hours post experiment. The height of the histograms corresponds to the mean percentage change in AKD, which is also presented numerically. The error bars represent the corresponding standard deviations. 'S' indicates that the reduction in AKD was significantly different from zero ($P < 0.05$). *Statistically significant differences ($P < 0.05$) between the experimental and control eyes, immediately after the experiment; (○) statistically significant differences ($P < 0.05$) between the experimental and control eyes, 2 hours post experiment.

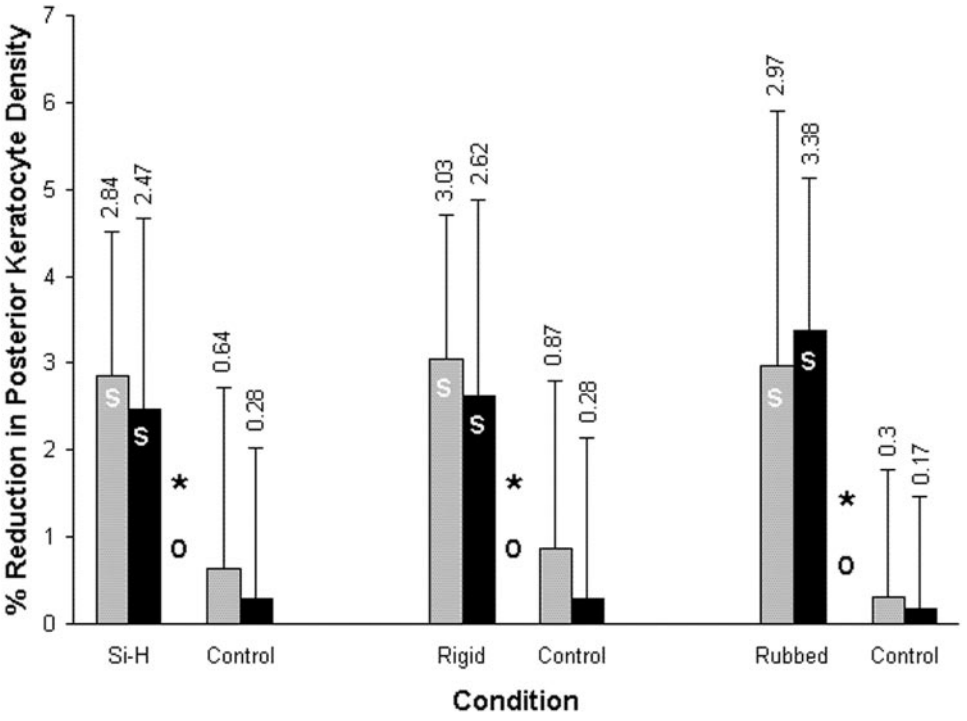

FIGURE 6. The percentage change in PKD relative to baseline in all eye groups in the three experimental sessions. The remainder of the description is the same as for AKD in Figure 5.

samples. EGF concentration, EGF rate of release, IL-8 concentration, and IL-8 rate of release were significantly higher in the tear samples collected from the rubbed eyes than in the samples collected from the control eyes ($F = 8.39$, $P = 0.006$; $F = 24.95$, $P < 0.001$; $F = 5.35$, $P = 0.026$; and $F = 11.65$, $P = 0.002$, respectively).

DISCUSSION

Monitoring keratocyte density may represent a new paradigm for evaluating the effects of contact lens wear on the cornea. Bansal et al. (*IOVS* 1997;38:ARVO Abstract S674) reported that anterior keratocyte density in long-term daily wearers of soft, rigid, and PMMA lenses was 18% lower than in non-lens wearers. Jalbert and Stapleton[11] used confocal microscopy to demonstrate that anterior and posterior keratocyte densities in corneas of nine long-term wearers of extended-wear hydrogel lenses were lower (32% and 18%, respectively) than in nine age-matched non–lens-wearing control subjects. These investigators suggested that contact-lens–induced keratocyte loss could be attributed to hypoxic, cytokine-mediated, and mechanical effects. Efron et al.[12] conducted a prospective study of

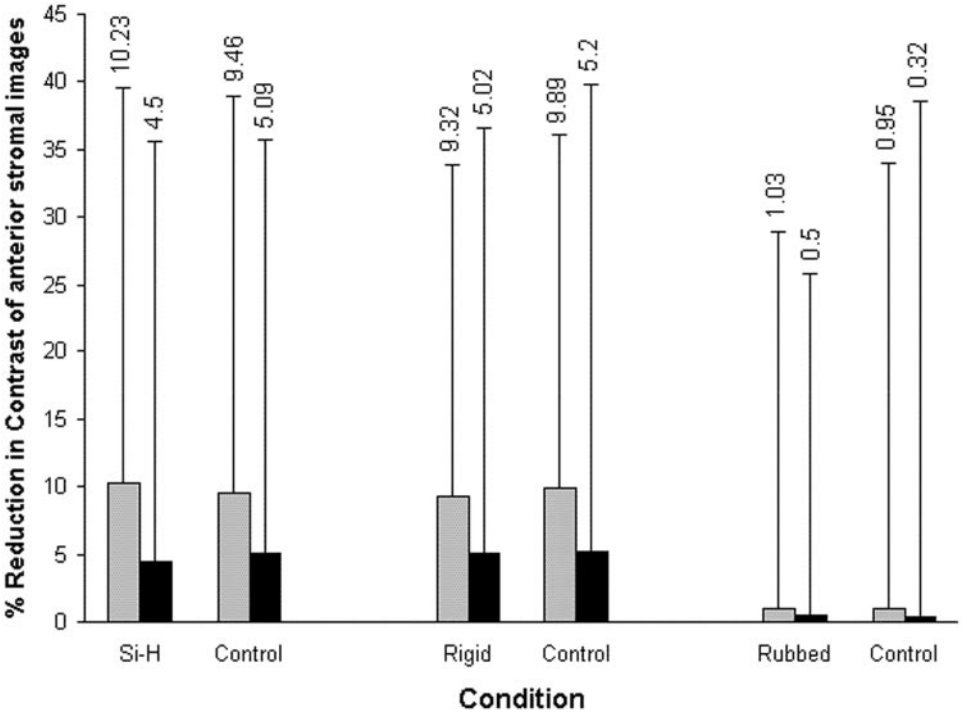

FIGURE 7. The percentage change in the contrast of anterior stromal images relative to baseline in all eye groups in the three experimental sessions. (□) The mean change in contrast immediately after the exposure period; (■) the change in contrast 2 hours post experiment. The height of the bars corresponds to the mean percentage change in contrast, which is also presented numerically. The error bars represent the corresponding standard deviations. The reduction in contrast of anterior stromal images was not significantly different from zero in any of the eye groups, at any of the three experimental sessions. No statistically significant differences were found for the percentage change in the contrast of anterior stromal images, between the experimental and control eyes both immediately after the experiment and 2 hours post experiment.

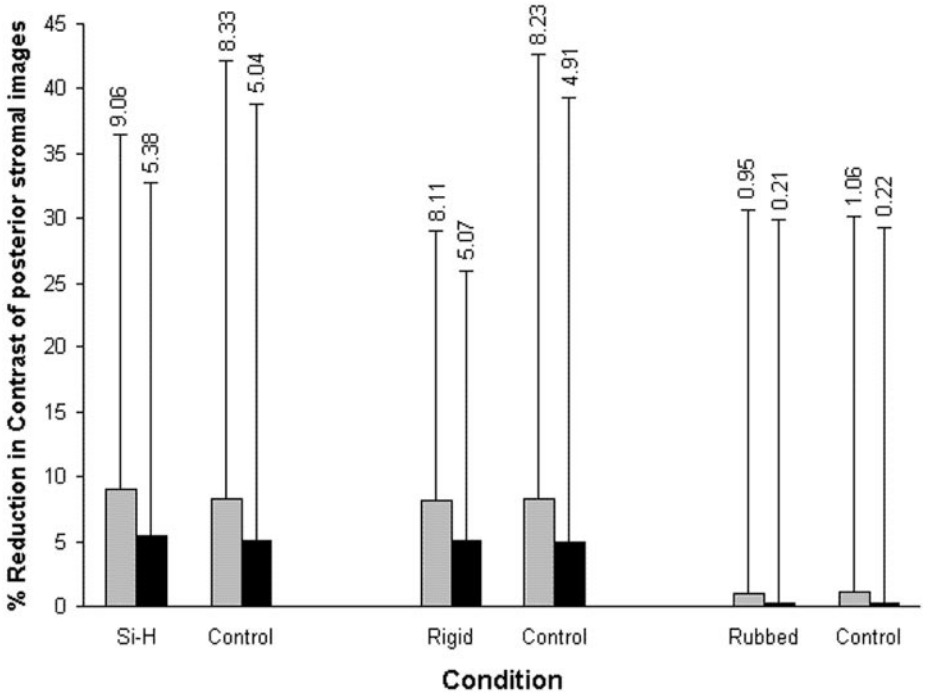

FIGURE 8. The percentage change in the contrast of posterior stromal images relative to baseline in all eye groups in the three experimental sessions. The remainder of the description is the same as for the contrast of anterior stromal images in Figure 7.

the effects of extended contact lens wear on keratocyte populations, which accounted for the effects of hypoxic edema on the determination of keratocyte density.[14] They compared the performance of a hydrogel lens of relatively low oxygen performance fitted to one eye with that of a silicone hydrogel lens of relatively high oxygen performance fitted to the fellow eye. The corneal thickness changes observed were consistent with the relative oxygen performances of the two lens types; however, the percentage reduction in PKD was similar for both lens types. The results of this study indicate that hypoxia and edema are not of etiological significance in lens-induced reduction in PKD. However, a subsequent study by Patel et al.[13] showed that long-term daily contact lens wear does not cause any alterations in keratocyte densities. These researchers compared the keratocyte densities in various depths of the corneal stroma between 20 daily contact lens wearers of any type of lens and 20 age-matched control subjects. Their results demonstrated that corneal thickness was similar in lens-wearing and control subjects, keratocyte densities were not altered by contact lens wear, and the contrast between cells and background was similar in lens-wearing and control subjects.

The present laboratory studies were designed to investigate the effects of short-term contact lens wear and mechanical stimulation of the cornea on keratocyte populations, after accounting for the potential confounding effects of edema on the evaluation of keratocyte density. This was achieved by exposing both the lens-wearing and control eyes to an anoxic environment, which would obviate the effects of edema when assessing keratocytes. As expected, the percentage increase in corneal thickness was similar in the experimental and control eyes. However, there was a greater reduction in both AKD and PKD in the Si-H- and the rigid-lens–wearing eyes compared with the control eyes, both immediately after the experiment and 2 hours post experiment. The reduction in both AKD and PKD was similar in the Si-H- and the rigid-lens–wearing eyes, indicating that the two types of lenses have effects of almost equal magnitude on the keratocyte population. We also demonstrated that 2 hours post experiment the reduction in AKD and PKD is still evident, indicating that no recovery is taking place within 2 hours.

The explanation of the discrepancy between our results and those reported by Patel et al.[13] is unclear. They presented the range of minimum detectable differences, due to the small sample sizes in each group in their study, and this could have limited the power of their statistical analysis. In addition, it is not clear why they performed ultrasound pachometry and

TABLE 4. Descriptive Statistics for EGF and IL-8 Concentrations and Rates of Release in the Tear Samples of the Rubbed and the Control Eyes

	Rubbed Eyes				Control Eyes			
	EGF Concentration (pg/mL)	EGF Rate of Release (pg/min)	IL-8 Concentration (pg/mL)	IL-8 Rate of Release (pg/min)	EGF Concentration (pg/mL)	EGF Rate of Release (pg/min)	IL-8 Concentration (pg/mL)	IL-8 Rate of Release (pg/min)
Number of observations	20	20	20	20	20	20	20	20
Mean	1763.7	380.4	534.8	102.1	844.5	92.7	422.9	52.1
SD	1205.2	247.9	207.6	61.8	748.1	69.6	60.2	21.6
Median	1560.6	360.4	508.6	95.0	619.7	79.2	1408.9	46.3
Maximum	4278.1	855.6	894.6	261.6	2974.8	241.2	538.0	127.1
Minimum	330.5	54.0	125.5	12.6	157.8	9.8	341.4	33.1

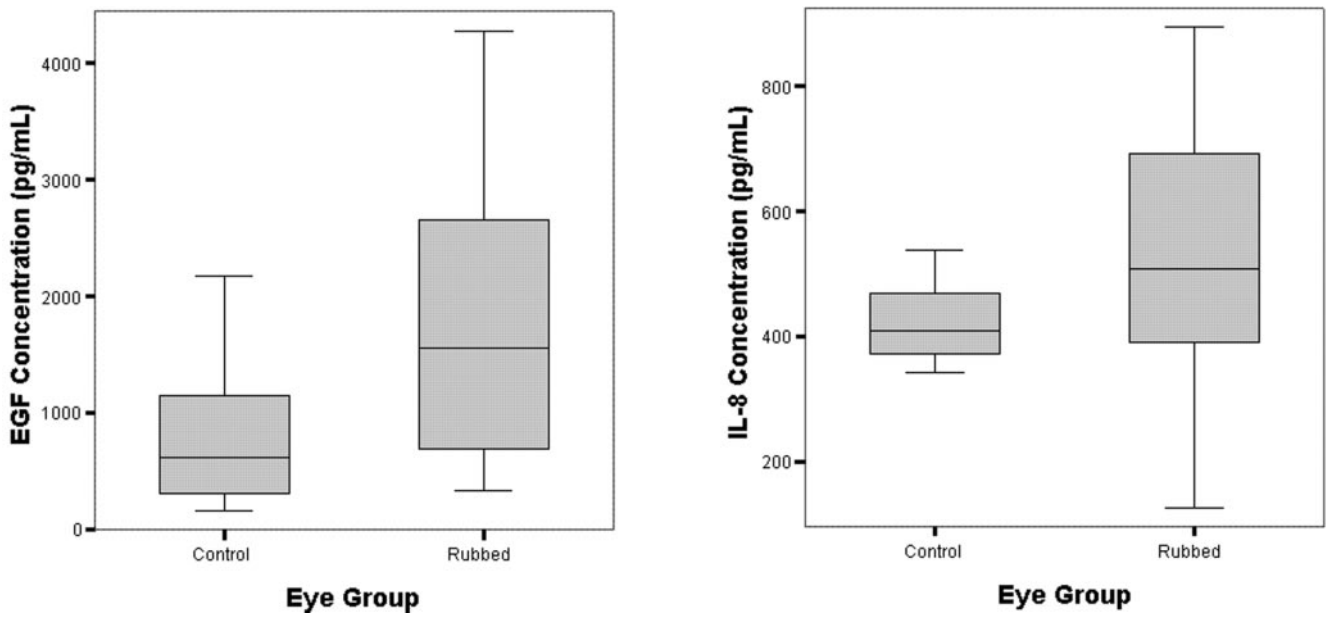

FIGURE 9. EGF concentration (*left*) and IL-8 concentration (*right*) in the tear fluid samples collected from the rubbed and control eyes. *Shaded box*: interquartile range (50% of the values); *whiskers*: the highest and lowest values; *horizontal line*: median. EGF and IL-8 concentrations were significantly greater in the samples collected from the rubbed eyes compared with the samples collected from the control eyes, ($F = 8.39$, $P = 0.006$) and ($F = 5.35$, $P = 0.026$), respectively.

confocal microscopy 12 to 24 hours after contact lens removal, after which time any acute edema would have resolved. The contrast of confocal images was decreased under hypoxic conditions. This degradation of image quality could make keratocytes more difficult to detect. Nevertheless, the decrease in contrast was similar in the experimental and control eyes at all time points. The decrease in contrast could hamper our ability to observe keratocytes and could create an artifact that might account for the slight decrease in AKD and PKD observed in the control eyes. These results on the contrast of confocal images are in agreement with those presented by Patel et al.[13,22]

To induce a mechanical effect on the cornea in the absence of a contact lens, we asked the subjects to slightly rub one eye only and compared the keratocyte densities in the rubbed and control eyes. We observed a greater reduction in both AKD and PKD in the rubbed compared with the control eyes. Although any mechanical effect induced by contact lens wear may be less severe than eye rubbing, we found that the reduction in both AKD and PKD were similar in the Si-H- and the rigid-lens–wearing eyes and the rubbed eyes. We cannot discount the possibility that the mechanical effect of eye rubbing was related to microtrauma of the internal structures and palpebral conjunctiva of the eyelid, as well as to microtrauma in the corneal epithelium. However, when a contact lens is fitted to the cornea, interference and alteration in the normal physiology of the palpebral conjunctiva also occur.

Keratocyte loss has been reported in response to corneal epithelial debridement,[23–25] either alone or in conjunction with refractive surgical procedures.[26–28] These studies suggest that the immediate disappearance of anterior keratocytes in response to epithelial injury is mediated by apoptosis. It was hypothesized that keratocyte apoptosis is directly related to epithelial injury and that this response is mediated by cytokines, growth factors, and other inflammatory mediators. These components may be derived from the lacrimal gland, conjunctival vessels, corneal cells, conjunctival cells, and other sources. Contact-lens–induced trauma to the epithelium could result in increased release of inflammatory mediators, facilitating the loss of corneal fibroblasts. Similarly, keratoconus has been associated with eye rubbing.[29] Persistent rubbing of the eye would be likely to produce corneal epithelial trauma and increase the release of IL-1 and other cytokines.[30]

In this study, we found that EGF and IL-8 concentrations were greater in the tear fluid samples collected from the rubbed eyes than in the samples obtained from the control eyes. EGF has been shown to be a constant component of normal human tear fluid.[17,31] The expression of EGF has been found to be upregulated in the lacrimal gland shortly after epithelial injury.[32] Similarly, IL-8 is a constant component of the normal human tear fluid.[33] IL-8 concentration in the tear fluid has been found to be higher in contact lens wearers than in control subjects.[34] The specific role of EGF and IL-8 in corneal wound healing is not very clear. It seems likely that EGF, IL-8, and other epithelium-modulating cytokines regulate proliferation, migration, and differentiation during the early wound-healing period until myofibroblasts or corneal fibroblasts repopulate the anterior stroma.[32]

HGF was not detected in any of the tear samples; however, it has been demonstrated that HGF is a constant component of the tear fluid.[35] Our inability to detect HGF could be explained by the relatively low sensitivity of the ELISA—the minimum detectable dose of HGF was 40 pg/mL—and/or the use of an inappropriate dilution factor.

It has been shown that quiescent keratocytes undergo a series of phenotypic changes during the wound-healing process. Ultrastructural evaluation of keratocytes adjacent to the wound has shown a clear transition from normal keratocytes to activated keratocytes containing prominent Golgi and extensive rough endoplasmic reticulum.[36] Inflammatory cells, such as macrophages, apparently play an essential role in wound healing.[37] Confocal microscopy identified these cells as oval, highly reflective cells, located in the subepithelial area.[38] Kaufman et al.[39] observed hyper-reflective keratocyte nuclei in the corneal stroma after 10 minutes of contact lens wear. The number and brightness of the highly reflective keratocyte nu-

clei decreased after 30 minutes of lens wear,[39] which may explain why we did not observe any hyper-reflective nuclei in our study of 2 hours' duration. The presence of hyper-reflective nuclei may be an acute transient change that occurs in the corneal stroma immediately after lens wear, but subsides after 30 minutes of lens wear.

One of the most important shortcomings of our study was that confocal microscopic observations were limited to the central cornea and to the anterior and posterior stroma. Evaluation of keratocyte density at the peripheral cornea and at the whole depth of corneal stroma is essential to validate that the observed drop in keratocyte density is due to a real loss of cells and not to a migration of cells either toward the peripheral cornea or along the anterior–posterior axis.

Further studies are needed to document and understand fully the exact mechanism of lens-induced keratocyte loss and to identify the vast number of inflammatory mediators that may play a role in this phenomenon.

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